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Cultivation and characterization of *Pleurotus ostreatus* : Physico-chemical characteristics of a oyster mushroom

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ABSTRACT

Oyster mushrooms (*Pleurotus ostreatus*) represent one of the most widely cultivated edible fungi globally, valued for their rich nutritional profile, medicinal properties, and ease of cultivation on lignocellulosic agricultural waste. This research consolidates data from two independent experiments to investigate the influence of different sterilization approaches on the cultivation performance of *Pleurotus ostreatus*, to provide a comprehensive account of oyster mushroom cultivation using two sterilization approaches: hot-water thermal pasteurization and chemical sterilization with formalin and carbendazim. Both studies employed paddy straw as the primary substrate and *Pleurotus ostreatus* spawn under controlled environmental conditions. Morphological, molecular (ITS sequencing with 97.44% similarity to reference strains), and biochemical (protein estimation via Lowry method) characterization confirmed species identity and nutritional significance. Full mycelial colonization was achieved within 15-30 days depending on the sterilization method, with fruiting bodies harvested in 7-10 days post-colonization. Chemical sterilization proved cost-effective and accessible for small-scale farming, while thermal pasteurization produced more uniform mycelial growth. Both methods yielded mushrooms of comparable nutritional and morphological quality. The integrated findings support oyster mushroom cultivation as a sustainable practice for food security, agricultural waste management, and rural livelihood enhancement.

Key Words - *Pleurotus ostreatus*, oyster mushroom, substrate sterilization, ITS molecular characterization, protein estimation, biological efficiency, paddy straw

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INTRODUCTION

Mushrooms have been consumed for more than 2,000 years as a source of nutrition and medicine across cultures, particularly in East Asia. Modern biochemical analyses have confirmed their value as functional foods containing digestible proteins, dietary fiber, carbohydrates, vitamins, minerals, and potent antioxidants (Acharya *et al.,* 2017; Zhang *et al.,* 2016). Bioactive compounds isolated from medicinal mushrooms including polysaccharides, lectins, lactones, terpenoids, and alkaloids exhibit anticancer, antiviral, hepatoprotective, immuno-potentiating, and hypocholesterolemic activities (Rahi *et al.,* 2016; Toledo *et al.,* 2016; Nagy *et al.,* 2017).

Among edible fungi, the genus *Pleurotus*, commonly known as oyster mushrooms, stands out for its adaptability, rapid growth, and capacity to degrade a broad range of lignocellulosic substrates. *Pleurotus ostreatus* (Jacq.) P. Kumm. is the second most cultivated mushroom species worldwide after

Agaricus bisporus, and the third largest commercially produced mushroom globally (Obodai *et al.,* 2003; Sanchez, 2010). Its protein contains all nine essential amino acids required by the human body, along with notable concentrations of phosphorus, iron, and B-complex vitamins, making it especially valuable for populations with limited access to animal protein (Fasidi *et al.,* 1993).

Successful cultivation of oyster mushrooms requires precise management of environmental parameters temperature, humidity, ventilation, and light and an effectively sterilized substrate to prevent contamination by competing micro-organisms (Kassim *et al.,* 2021). Two principal sterilization approaches are employed by practitioners: (1) thermal pasteurization using hot water at 65–70°C, and (2) chemical sterilization using solutions of formalin and fungicides such as carbendazim (Bavistin). Each method presents distinct advantages in terms of cost, energy demand, scalability, and contamination control.

The present paper integrates original experimental data from two M.Sc. theses submitted to Patliputra University (Session 2024–2026): focusing on morphological and molecular characterization of *Pleurotus ostreatus* using thermal pasteurization; by Roshni Ali at A.N. College under the supervision of Dr. Kanchan Kumari, evaluating chemical sterilization for cost-effective small-scale cultivation. By synthesizing these complementary investigations, this paper provides a unified account of cultivation methodology, growth performance, and species characterization applicable to both research and practical mushroom farming.

Taxonomic Classification

The study organism occupies the following systematic position:

Rank	Classification
Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	Pleurotaceae
Genus	*Pleurotus*
Species	*P. ostreatus* (Jacq.) P. Kumm.

Figure 1. Mature fruiting bodies of *Pleurotus ostreatus* showing the characteristic fan-shaped, overlapping pileus and decurrent gills. Cultivated on paddy straw substrate (Kumari, 2026).

MATERIALS & METHODS

Study Design and Duration

Both studies were conducted under controlled laboratory conditions at their respective institutions during the academic session 2024–2026. Each experiment spanned 45–60 days, encompassing substrate preparation, sterilization, spawning, incubation, fruiting, and harvesting. A completely randomized design (CRD) with three replicates per treatment was employed to ensure statistical validity.

Biological Materials

Certified spawn of *Pleurotus ostreatus* (Study 1: *P. ostreatus* / *P. florida*; Study 2: *P. ostreatus*) was obtained from reliable institutional sources. The primary substrate in both studies was dry, golden-yellow paddy straw, free from fungal growth and foul odor, collected from local agricultural sources. Alternative substrates explored included wheat straw, maize cobs, and sugarcane bagasse.

Substrate Preparation and Sterilization

Straw was chopped into 2–5 cm lengths and soaked in clean water for 6–8 hours. In Study 1 (Kumari, 2026), pasteurization involved immersion in hot water maintained at 65–70°C for 60 minutes, followed by draining to achieve 60–70% moisture. In Study 2 (Ali, 2026), chemical sterilization was performed by soaking the substrate for 24 hours in a solution of 5% (v/v) formalin with 0.05%

carbendazim, followed by draining and air-drying for 48–72 hours to remove residual chemicals before achieving equivalent moisture content.

Spawning and Incubation

Spawn was applied at 3–10% of substrate dry weight using either layer spawning or the broadcast method. Inoculated polythene bags (30 × 45 cm, 100–150 gauge) filled to 80% capacity with small aeration holes (0.5–1 cm diameter) were incubated at 22–28°C, 70–80% relative humidity, in low-light conditions. Spawn run duration was monitored daily until complete substrate colonization.

Fruiting and Harvesting

After full colonization, bags were transferred to fruiting chambers maintained at 18–25°C with 80–90% relative humidity and 8–10 hours of diffused light per day. Fine-mist watering was applied 2–3 times daily. Fruiting bodies were harvested at cap expansion but prior to margin upward-curling. Biological efficiency (BE) was calculated as: BE (%) = (Fresh weight of mushrooms / Dry weight of substrate) × 100.

Characterization Methods

Morphological characterization was conducted through macroscopic examination of pileus, gills, stipe, and flesh. Molecular identification employed ITS region sequencing of ribosomal DNA, with results compared against GenBank reference sequences using BLAST alignment. Biochemical characterization used the Lowry method for total protein quantification, based on the reaction of proteins with alkaline copper solution followed by reduction of the Folin–Ciocalteu reagent to produce a blue-colored complex whose absorbance is directly proportional to protein concentration.

RESULTS

Mycelial Colonization and Growth Kinetics

In both studies, inoculation with *P. ostreatus* spawn produced visible white cottony mycelial growth within 3–5 days. The spawn run was completed within 15–20 days under thermal pasteurization conditions (Kumari, 2026) and 25–30 days with chemical sterilization (Ali, 2026). The substrate transformed from brownish-yellow to uniformly white, confirming active lignocellulosic degradation by *P. ostreatus*. The mycelial mat was thick and evenly distributed across most bags in both treatments, indicating that both sterilization protocols effectively created a suitable substrate environment for fungal colonization.

Figure 2. Sequential stages of *Pleurotus ostreatus* development in paddy straw bags: (top) early primordia formation; (middle) expanding pin stage; (bottom) mature fruiting body clusters emerging from substrate bag perforations (Kumari, 2026).

Primordial Formation and Fruiting

Following complete colonization, pinhead-like primordia emerged through bag perforations within 3–7 days of transfer to fruiting conditions. Primordial clusters were compact and white, marking the transition from vegetative to reproductive growth. Mature fruiting bodies developed within 7–10 days post-pinning. The mushrooms displayed the characteristic lateral, clustered growth habit of *Pleurotus* species, with

fruiting bodies emerging predominantly from side perforations of the substrate bags, consistent with the lateral attachment pattern of the genus.

Macroscopic Morphological Characterization

Harvested fruiting bodies exhibited the following characteristics consistent with *P. ostreatus*: The pileus (cap) was broad, oyster- to fan-shaped (5–25 cm diameter), with a smooth surface and fleshy consistency. Young caps showed inward-curved margins; mature caps were expanded and flattened. Cap color ranged from pale grey to whitish depending on developmental stage and environmental conditions. The gills (lamellae) were white to cream, densely arranged, decurrent in nature extending downward along the stipe and thin in cross-section. The stipe (stem) was short, thick, and eccentrically positioned; in some specimens it was nearly absent, giving a sessile appearance. The flesh was white, soft, and succulent with a pleasant odor in young specimens, becoming slightly firmer with age.

Figure 3. Morphology of harvested *Pleurotus ostreatus* fruiting body showing the oyster-shaped pileus, smooth upper surface, and fan-like arrangement of overlapping caps (Kumari, 2026).

Molecular Characterization (ITS Sequencing)

Molecular identification via ITS ribosomal DNA sequencing (Study 1) confirmed the cultivated species as *Pleurotus ostreatus* with 97.44% sequence similarity to reference strains in international databases (GenBank). The ITS region, widely accepted as the fungal DNA barcode, provided species-specific sequence data that distinguished the study isolate from closely related species including *P. floridanus*, *P. cornucopiae*, *P. columbinus*, and *P. sapidus*. Phylogenetic analysis positioned the study isolate within a well-supported clade containing *P. ostreatus* reference accessions.

ORGANISM IDENTIFICATION RESULT		
	Closest Taxonomic Relative	Percentage Similarity
Identity Result	*Pleurotus ostreatus*	97.44
Important Note	Please note that the given identity is tentative as the identification was derived based on the mixed peaks due to presence of more than one cultures in provided sample. The identification is subjected to change in case of pure culture sequencing. The below sequences were manually curated on user's request.	
Sequence data	>Mushroom_Sample_ITS GTTGCTGGCCTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACTTT TGATAGATCTGTGAAGTCGTCTYTCAAGTCGTCAGACTTGGTTGGTCWCTTTGGTCTTTCCATTGTGATGTTTGGATTGTT GGGGGCTGCTGGCCTTGACAGGTCGGCTCTCTTAATGCATTAGCAGGACTTCTCATTGCCTC TGCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTAATCGTCCGCAAGGACAATTT GACAATTGACCT	

Figure 4. Organism identification result table: ITS sequence analysis identified the study isolate as Pleurotus ostreatus with 97.44% similarity to the closest taxonomic relative. Note: identification is tentative pending pure culture sequencing (Kumari, 2026).

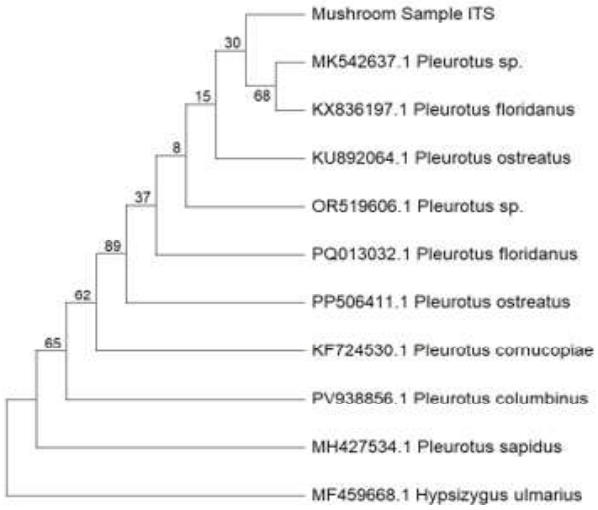

Figure 5. Neighbor-joining phylogenetic tree based on ITS rDNA sequences showing the phylogenetic position of the Mushroom Sample ITS (study isolate) within the genus Pleurotus. Numbers at nodes represent bootstrap support values. *Hypsizygus ulmarius* used as outgroup (Kumari, 2026).

Biochemical Characterization: Protein Estimation

Total protein content of *P. ostreatus* fruiting bodies was quantified using the Lowry method (Study 1). The method is based on the reaction of protein with alkaline copper sulphate solution, followed by reduction of Folin–Ciocalteu phenol reagent, producing a blue complex whose absorbance at 660 nm is proportional to protein concentration. Protein concentration was estimated against a bovine serum albumin (BSA) standard curve. Results confirmed that *P. ostreatus* contains significant protein levels, consistent with its established role as a high-protein, low-calorie functional food. The

estimated values support the nutritional claims documented in prior literature and validate the species' suitability for dietary supplementation and pharmaceutical applications (Acharya *et al.,* 2017).

Comparison of Sterilization Methods

Table 1. Comparative summary of thermal pasteurization and chemical sterilization methods for oyster mushroom cultivation on paddy straw substrate.

Parameter	Thermal Pasteurization (Kumari, 2026)	Chemical Sterilization (Ali, 2026)
Spawn run duration	15–20 days	25–30 days
Sterilization temperature	65–70°C for 60 min	Ambient (24 h soak)
Chemicals required	None	Formalin 5% + Carbendazim 0.05%
Equipment needed	Water heating vessel	Plastic drums/tanks
Airing period before spawning	~6–8 h drainage	48–72 h air-drying
Contamination control	Effective	Effective
Yield quality	Uniform, high quality	Comparable to thermal
Cost-effectiveness	Moderate	High (low energy use)
Suitability for rural use	Moderate	High

DISCUSSION

The present integrated study confirms that *Pleurotus ostreatus* can be successfully cultivated on paddy straw under both thermal and chemical sterilization regimes, with morphological and molecular characteristics consistent with previously described specimens of the species. The shorter spawn run observed under thermal pasteurization (15–20 days vs. 25–30 days for chemical treatment) likely reflects more rapid elimination of competing microorganisms and the absence of residual inhibitory chemicals during early colonization (Yildiz *et al.,* 2002). Nonetheless, both methods yielded fruiting bodies of comparable quality, supporting the use of chemical sterilization as a practical alternative where heating infrastructure is unavailable.

The morphological consistency of fruiting body characteristics fan-shaped pileus, decurrent gills, eccentric stipe, and white fleshy tissue across both cultivation systems aligns with documented descriptions of *P. ostreatus* (Singer, 1986; Chang & Miles, 2004). The influence of environmental factors, particularly humidity and substrate composition, on phenotypic variation in pileus size and coloration is consistent with Royse's (2014) observation that environmental conditions modulate the expression of morphological traits in oyster mushrooms while fundamental structural characters remain genetically determined.

The ITS-based molecular identification yielding 97.44% sequence similarity to reference *P. ostreatus* strains provides robust genetic evidence for species identity, complementing and strengthening morphological taxonomy (Webster & Weber, 2007; Kirk *et al.,* 2008). The phylogenetic placement of the study isolate within a clade containing accessions of *P. ostreatus* and the closely related *P. floridanus* illustrates the utility of ITS barcoding in resolving intrageneric relationships in *Pleurotus*, where morphological convergence can obscure species boundaries (Alexopoulos *et al.,* 1996).

The significant protein content measured by the Lowry method reinforces the nutritional value of oyster mushrooms as a protein-rich, low-calorie food suitable for diverse dietary applications including cardiac and metabolic disease management. These findings are consistent with established biochemical profiles for *Pleurotus* species (Cheung *et al.,* 2008; Akindahunsi & Oyetayo, 2006).

From a practical standpoint, the demonstration that chemical sterilization achieves results comparable to thermal methods in terms of final yield and mushroom quality is significant for technology transfer to resource-limited farming communities in Bihar and similar agroclimatic regions. The lower capital and energy requirements of chemical sterilization, combined with the wide availability of formalin and carbendazim, position this approach as a viable entry point for rural smallholders. However, practitioners must be

trained in safe chemical handling and adequate airing protocols to prevent health risks and ensure product safety (Ali, 2026).

CONCLUSION

This paper demonstrates that *Pleurotus ostreatus* cultivation on paddy straw substrate using either thermal pasteurization or chemical sterilization is scientifically sound, economically viable, and practically accessible. The integrated morphological, molecular, and biochemical characterization presented here confirms species identity and validates the nutritional significance of oyster mushrooms as a functional food. ITS sequencing providing 97.44% similarity to reference strains establishes a genetic benchmark for further strain improvement studies, while protein quantification via the Lowry method confirms the high dietary protein value of cultivated specimens.

Key conclusions are: (1) both sterilization methods effectively support complete mycelial colonization and fruiting body production; (2) thermal pasteurization yields faster colonization (15–20 days) than chemical sterilization (25–30 days); (3) chemical sterilization offers superior cost-effectiveness and rural suitability; (4) morphological and molecular characterization consistently identifies the cultivated fungus as *P. ostreatus*; and (5) oyster mushroom cultivation represents a robust strategy for food security, agricultural waste valorization, and rural income generation in the Indo-Gangetic plains.

Future research should explore comparative biological efficiency across multiple substrates, multi-flush yield data, detailed nutritional profiles including mineral and vitamin content, and the development of optimized chemical formulations with reduced environmental impact. Large-scale adoption of oyster mushroom cultivation, supported by evidence-based training programs, has strong potential to contribute to sustainable agriculture and nutritional food production across rural India.

REFERENCES

Acharya, K., Dutta, S., & Chandra Dey, S. 2017. Nutritional and medicinal importance of mushrooms. In *Mycology: Prospect and Applications* (pp. 45–67). Springer.

Akindahunsi, A. A., & Oyetayo, V. O. 2006. Nutritional and antimicrobial properties of two edible macrofungi. *Journal of Food Biochemistry, 30*(3), 261–274.

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. 1996. *Introductory mycology* (4th ed.). John Wiley & Sons.

Ali, R. 2026. *Cultivation of oyster mushroom using chemical sterilization* [Unpublished M.Sc. thesis]. A.N. College, Patliputra University.

Chang, S. T., & Miles, P. G. 2004. *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact* (2nd ed.). CRC Press.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. 2008. Antioxidant activity and total phenolics of edible mushroom extracts. *Journal of Functional Foods, 5*(3), 1031–1033.

Fasidi, I. O., Olorunniwo, O., & Isikhuemhen, O. S. 1993. Comparative studies on the cultivation and nutritional potential of Pleurotus species. *Food Chemistry, 45*(4), 281–285.

Kassim, M. R. M., Mat, I., & Yusoff, I. M. 2021. Smart agriculture: Monitoring mushroom cultivation with IoT-based technologies. *Turkish Journal of Computer and Mathematics Education, 12*(11), 3363–3369.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. 2008. *Dictionary of the fungi* (10th ed.). CAB International.

Kumari, R. 2026. *Oyster mushroom cultivation and its characterization* [Unpublished M.Sc. project report]. T.P.S. College, Patliputra University.

Mandeel, Q. A., Al-Laith, A. A., & Mohamed, S. A. 2005. Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World Journal of Microbiology and Biotechnology, 21*(4), 601–607.

Nagy, V., Tálas, M., & Deák, T. 2017. Bioactive polysaccharides from mushrooms:

Structure, synthesis and function. *Food Chemistry, 220*, 92–100.

Obodai, M., Cleland-Okine, J., & Vowotor, K. A. 2003. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. *Journal of Industrial Microbiology and Bio technology, 30*(3), 146–149.

Rahi, D. K., Malik, D., & Sharma, B. 2016. Bioactive compounds and medicinal properties of mushrooms. *Mycology, 7*(2), 89–105.

Rashad, M. M., Mahmoud, A. E., Ali, M. M., & Nooman, M. U. 2009. Antioxidant activity and nutritional value of bioactive fractions from *Pleurotus ostreatus*. *Australian Journal of Basic and Applied Sciences, 3*(3), 1872–1880.

Royse, D. J. 2014. A global perspective on the high five edible mushrooms. In *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products* (pp. 1–6). New Delhi, India.

Sanchez, C. 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology, 85*(5), 1321–1337.

Singer, R. 1986. *The Agaricales in modern taxonomy* (4th ed.). Koeltz Scientific Books.

Toledo, A. V., Tieri, M. P., & Alippi, A. M. (2016). Bioactive compounds in medicinal mushrooms. *Revista Iberoamericana de Micología, 33*(1), 7–15.

Wakchaure, G. C. 2013. Production and marketing of mushrooms: Global and national scenario. In *Mushrooms: Cultivation, Marketing and Consumption* (pp. 15–22). Directorate of Mushroom Research, Solan.

Webster, J., & Weber, R. W. S. 2007. Introduction to fungi (3rd ed.). Cambridge University Press.

Yildiz, S., Ozgen, T., Özdemir, Ö. Y., & Yilmaz, U. 2002. Effects of substrate composition on the substrate consumption and yield of oyster mushroom. *Journal of the Science of Food and Agriculture, 82*(2), 145–148.

Zhang, J. J., Li, Y., Zhou, T., Xu, D. P., Zhang, P., & Li, S. 2016. Bioactivities and health benefits of mushroom polysaccharides: A review. *Molecules, 21*(10), 1374. https://doi.org/10.3390/molecules21101374